JOURNAL OF VIROLOGY, Dec. 2007, p. 13916–13921 Vol. 81, No. 24
0022-538X/07/$08.00+0 doi:10.1128/JVI.01585-07

NOTES

Adhesion Molecule Interactions Facilitate Human Immunodeficiency Virus Type 1-Induced Virological Synapse Formation between T Cells[▽]

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Received 20 July 2007/Accepted 24 September 2007

Human immunodeficiency virus type 1 (HIV-1) can spread between CD4$^+$ T cells by using a virological synapse (VS). The VS assembly is a cytoskeleton-driven process dependent on HIV-1 envelope glycoprotein (Env)-receptor engagement and is hypothesized to require adhesion molecule interactions. Here we demonstrate that leukocyte function-associated antigen 1 (LFA-1), intercellular adhesion molecule 1 (ICAM-1), and ICAM-3 are enriched at the VS and that inhibition of these interactions influences conjugate formation and reduces VS assembly. Moreover, CD4$^+$ T cells deficient in LFA-1 or with modified LFA-1 function were less able to support VS assembly and cell-cell transfer of HIV-1. Thus, cognate adhesion molecule interactions at the VS are important for HIV-1 spread between T cells.

Human immunodeficiency virus type 1 (HIV-1) can disseminate within and between hosts by cell-free and cell-associated means (18, 21, 25). A supramolecular structure, termed a virological synapse (VS), which mediates spread between infected (effector) and uninfected (target) T cells (17, 19), has been described for both HIV-1 and human T-cell leukemia virus type 1 (HTLV-1). The HIV-1 VS was so named because of partial functional homology with the immunological synapse (IS) that forms between immune cells. Immune cells do not constitutively form stable contacts but can do so during IS or VS formation (21). Assembly of the HIV-1 T-cell VS requires engagement of the HIV-1 Env surface subunit gp120, expressed on the effector cell, with its cellular receptors CD4 and CXCR4 on the target cell (19). Further recruitment of receptors and HIV-1 proteins to the conjugate interface is a cytoskeleton-dependent process in both target and effector T cells (19, 20). Like the IS (22), the VS is characterized by clustering of adhesion molecules such as the integrin leukocyte function-associated antigen 1 (LFA-1, also known as α_L β_2 or CD11a/CD18) at the effector–target cell interface (19), which is hypothesized to contribute to the formation of a stable adhesive junction.

Although adhesion interactions influence HIV-1 infection by serving as attachment cofactors for cell-free virions (4, 9, 11, 12, 15, 24, 27, 30–32), their contribution to cell-cell dissemination has been little studied. Integrins have been implicated in cell-cell transmission of HIV-1 from dendritic cells (DCs) to T cells via LFA-1 and DC-SIGN (DC-specific intercellular adhesion molecule 3 [ICAM-3]-grabbing nonintegrin), and their probable role in this setting is to maintain robust DC–T-cell contacts (13, 34). LFA-1 clusters at the VS in T-cell–T-cell interactions, but the identity of its ligands on opposing cells, their arrangement, and the functional contribution of such interactions to HIV-1 VS formation and cell-cell spread are undefined. Because LFA-1 is enriched at the VS, and in light of the importance of adhesion molecule interactions in both IS formation and cell-free HIV-1 infection, we investigated the hypothesis that adhesion interactions promote and/or maintain T-cell VS formation and cell-cell spread of HIV-1.

The cognate ligands of LFA-1 on T cells are ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50). Naïve and resting T cells are reported to express low levels of ICAM-1 and ICAM-2 (28) but high levels of ICAM-3 (8) constitutively, and following T-cell activation, ICAM-1 expression is upregulated (5). We used the Jurkat T-cell line infected with HIV-1_{IIIB} (Jurkat$_{IIIB}$ cells) as effectors and primary CD4$^+$ T cells as targets to analyze VS assembly and function, as previously described (19). To characterize adhesion molecule expression on Jurkat and primary CD4$^+$ T cells, we performed surface staining and flow cytometry with 10 different monoclonal antibodies (MAbs). Jurkat T cells and primary CD4$^+$ T cells were washed in cold fluorescence-activated cell sorter wash buffer (phosphate-buffered saline with 1% fetal calf serum and 0.01% sodium azide), and cells were incubated on ice for 1 h with as much as 20 μg/ml of the following antibodies against ICAM-1, ICAM-2, ICAM-3, and LFA-1: ICAM-1-specific clone LB-2 (BD Pharmingen, San Diego, CA); ICAM-2-specific clone BT-1 (Serotec, Oxford, United Kingdom); ICAM-3-specific clones 101-1D2 (Chemicon International, Temecula, CA) and BRIC79 (a gift from D. Anstee, Cell Adhesion Section, Bristol Institute for Transfusion Sciences, Bristol, United Kingdom); LFA-1 α_L (CD11a)-specific mouse ascites 25.3.1 (a gift from D. Olive, INSERM U119, Marseilles, France), clones L15 and

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▽ Published ahead of print on 3 October 2007.

TABLE 1. Flow cytometric analysis of adhesion molecule expression on $CD4^+$ T cells

Antigen	Antibody[a]	Blocking activity	Expression[a] on:	
			Primary $CD4^+$ T cells	Jurkat T cells
ICAM-1	LB-2	+	17.9	34
ICAM-2	BT-1	+	11.7	230
ICAM-3	101-1D2	−	94	28.4
	BRIC79	+	770	745.2
LFA-1 α_L	25.3.1	+	297.7	62.3
	L15	+	201.6	18.8
	TS2/4	−	214.3	32.5
	MHM24	−	15.2	1.7
LFA-1 β_2	MHM23	+	329	46.7
	L130	+	81.4	15.5
Control	IgG1		5.1	7.5
	IgG2a		3.9	7.6
	IgG2b		3.8	7.7
	Anti-mouse IgG-phycoerythrin		4.7	4.7

[a] Mean fluorescence intensity from three independent experiments after subtraction of background binding of the isotype-matched control antibodies. The mean fluorescence intensities of the controls are shown for reference.

TS2/4 (a gift from B. Joosten, Department of Tumor Immunology, University Medical Center, Nijmegen, The Netherlands), and MHM24 (provided by A. McMichael, MRC Human Immunology Unit, University of Oxford, Oxford, United Kingdom); and LFA-1 β_2 (CD18)-specific MHM23 (provided by A. McMichael) and clone L130 (BD Pharmingen). The cells were then incubated with phycoerythrin-conjugated anti-mouse immunoglobulin G (IgG) for 30 min on ice and fixed with 1% formaldehyde. Acquisition and analysis were performed using a Becton Dickinson FACSCalibur flow cytometer and CellQuest software. Primary $CD4^+$ T cells expressed low, intermediate, and high levels of ICAM-2, ICAM-1, and ICAM-3, respectively (Table 1). Lower expression of ICAM-1 than of ICAM-3 on primary T cells reflects the predominance of resting and naïve $CD4^+$ T cells in peripheral blood (5). Jurkat T cells expressed approximately twofold more ICAM-1 than primary $CD4^+$ T cells, reflecting their immortalized phenotype. ICAM-2 expression was low on primary cells but higher on Jurkat cells, whereas ICAM-3 expression, measured using BRIC79, was very high on both cell types. Expression of both the α_L and β_2 LFA-1 subunits was higher on primary cells than on Jurkat cells. We analyzed whether HIV-1 infection of T cells alters adhesion molecule expression by surface staining and flow cytometry of uninfected Jurkat cells or $Jurkat_{IIIB}$ cells using MAbs against ICAM-1, ICAM-2, ICAM-3, and LFA-1. No significant difference was observed with any of the MAbs tested (data not shown).

As with the IS, LFA-1 and the actin adaptor protein talin are recruited to the VS, and up to ~60% of VSs show polarization of LFA-1 at the cell-cell interface (19). However, since the presence of LFA-1 does not necessarily signify a functional role in adhesion, we examined whether the cognate ligands of LFA-1 (ICAM-1, ICAM-2, and ICAM-3) are similarly recruited to the VS. Figure 1A shows laser scanning confocal microscopy (LSCM) images taken through the center of conjugates incubated for as long as 1 h at 37°C and stained for Env, Gag, and adhesion molecules, as previously described (19). Inclusion of nonblocking MAbs against LFA-1, ICAM-2, and ICAM-3 during conjugate formation at 37°C had no effect on receptor localization (data not shown). By contrast, anti-ICAM-1 MAb LB-2 inhibits integrin binding and so was used post-conjugate formation. The recruitment of Env and Gag to the conjugate interface in $Jurkat_{IIIB}$ cells is characteristic of the HIV-1 T-cell VS, as is enrichment of LFA-1 (19). Although the strongest LFA-1 staining appears to be on the target cell, LFA-1 enrichment may also occur on the effector cell. Polarization of ICAM-3 to the interface was robust, and its colocalization with Env and Gag was apparent in 67% of conjugates with a VS, compared to 23% of conjugates where the cells were closely apposed but without a VS. By contrast, ICAM-1 staining was weaker, the recruitment of ICAM-1 to the VS was more limited, and fewer VSs showed interface-polarized ICAM-1 (38%, compared to 10% in the non-VS conjugates). As with LFA-1, ICAM-3 and ICAM-1 recruitment probably occurred on both effector and target T cells. ICAM-2 staining was very weak, and no enrichment at the VS was apparent (data not shown).

To determine if recruitment of LFA-1 and ICAMs to the VS had functional consequences, we used MAbs inhibitory (LB-2, BT-1, BRIC79, 25.3.1, L15, MHM23, and L130) and noninhibitory (TS2/4, 101-1D2, and MHM24) for LFA-1–ICAM interactions. Because primary $CD4^+$ T cells generally express higher levels of LFA-1 and ICAM-3 than Jurkat cells (Table 1), we preincubated the targets with saturating amounts of MAbs prior to mixing with effectors. Conjugate formation was arrested after 1 h by fixation and analyzed by LSCM. A typical HIV-1 VS, characterized by copolarization of HIV-1 Env and Gag on the effector cell with CD4 on the target cell, is shown in Fig. 1B (left). Inhibition of VS formation by blocking MAbs was defined by a loss of CD4 and Gag/Env polarization to the conjugate interface (Fig. 1B, right).

Randomly chosen low-power fields of conjugates formed in the presence or absence of MAb were imaged, allowing the percentages of target and effector cells forming conjugates and the percentage of conjugates containing a VS to be quantified (Table 2). A total of 5×10^5 fresh, primary $CD4^+$ target T cells were resuspended in RPMI medium–1% fetal calf serum and either left untreated or incubated with 40 μg/ml of antibodies against ICAM-1, ICAM-2, ICAM-3, or LFA-1 for 30 min at 37°C prior to mixing with $Jurkat_{IIIB}$ effector cells. Alternatively, primary cells were preincubated with 200 μM cyclic peptide cIBR (cyclo-1,12-Pen-PRGGSVLVTGC) or cLABL (cyclo-1,12-Pen-ITDGEATDSGC) (both kindly provided by T. J. Siahaan, University of Kansas). The cells were then incubated for 1 h at 37°C; stained for CD4, Env, and Gag; and examined by LSCM. Conjugates were defined as closely apposed pairs of cells containing at least one $CD4^+$ and one Env^+ or Gag^+ cell. Each conjugate pair was analyzed for cocapping of Env, Gag, and CD4 to the cellular interface. The results were similar to our previous observations (19): 19% of target cells formed conjugates with effector cells, and 46% of these conjugates evolved to a VS within 1 h. The α_L-specific blocking MAbs 25.3.1 and L15 did not significantly reduce conjugate formation but significantly inhibited VS formation

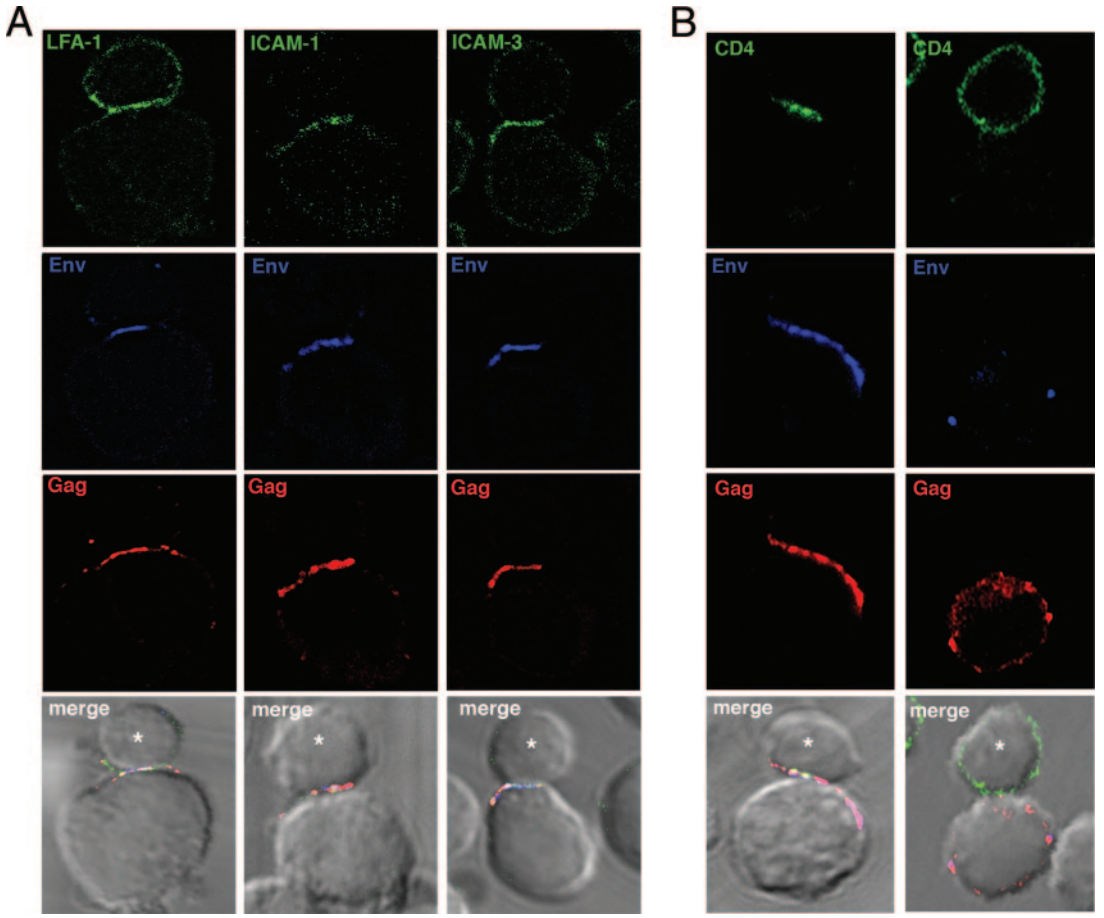

FIG. 1. Localization of LFA-1, ICAM-1, and ICAM-3 at the VS. (A) $CD4^+$ $CXCR4^+$ Jurkat CE6.1 cells (American Type Culture Collection) were maintained and infected with HIV-1 strain IIIB as described previously (19). A total of 5×10^5 $Jurkat_{IIIB}$ cells were mixed with an equal number of primary $CD4^+$ target T cells freshly isolated from peripheral blood (19) in RPMI medium supplemented with 1% fetal calf serum and were incubated on poly-L-lysine-coated coverslips for 1 h at 37°C to allow conjugates to form. Nonblocking human MAbs against HIV-1 Env (50-69) (blue) and either mouse anti-LFA-1 (TS2/4) (green) or anti-ICAM-3 (IC3/1; a gift from T. Springer, Harvard Medical School, Boston, MA) (7) (green) were included during this incubation. Alternatively, the cells were incubated with the anti-ICAM-1 MAb (LB-2) (green) postfixation. Conjugate evolution was arrested by fixation in cold 4% paraformaldehyde, and the cells were permeabilized in 0.1% Triton X-100–5% fetal calf serum for 20 min at room temperature, washed extensively in phosphate-buffered saline–1% bovine serum albumin, and stained with rabbit serum against the HIV-1 capsid protein Gag (red). Primary antibodies were detected with conjugated donkey anti-mouse, anti-human, and anti-rabbit secondary antibodies that were tested for the absence of interspecies reactivity (Jackson ImmunoResearch). Coverslips were mounted with ProLong antifade mounting solution (Molecular Probes), and confocal analysis was performed using a Bio-Rad Radiance 2000 MP LSCM. Images are single sections through the middle of a conjugate. Asterisks indicate target cells. Areas of colocalization appear white on the overlaid image. (B) A total of 5×10^5 primary $CD4^+$ target T cells that either were not pretreated or were pretreated for 30 min with adhesion molecule-specific MAbs were first mixed with an equal number of $Jurkat_{IIIB}$ cells in the presence of anti-Env MAb 50-69 (blue) and the noninhibitory anti-CD4 mouse MAb L120 (green) and then incubated on poly-L-lysine-coated coverslips for 1 h at 37°C to allow conjugates to form. Cells were then fixed in 4% formaldehyde at 4°C, permeabilized, stained for Gag (red), and analyzed by LSCM. (Left) VS formation in the absence of anti-adhesion molecule MAbs. (Right) Primary $CD4^+$ target T cells preincubated with the blocking anti-LFA-1 mouse MAb 25.3.1 prior to mixing with $Jurkat_{IIIB}$ cells. Images are single sections through the middle of a conjugate. The target cell is indicated with an asterisk. Areas of colocalization appear white on the overlaid image.

by 83% and 42%, respectively (Table 2). LFA-1 β_2-specific blocking MAbs MHM23 and L130 had differential effects: L130 significantly reduced conjugate and VS formation (40% and 63%, respectively), whereas MHM23 increased conjugate frequency ($P < 0.01$) but reduced VS formation by 89% ($P < 0.01$). Both anti-ICAM-1 and anti-ICAM-3 MAbs were inhibitory. LB-2 did not affect conjugate frequency but significantly reduced VS formation by 31%; BRIC79 significantly reduced conjugate frequency by 65% ($P < 0.01$) and VS formation by 70% ($P < 0.05$); and 101-1D2 inhibited VS formation by 27% ($P < 0.05$) without influencing conjugate frequency. By contrast, the anti-ICAM-2 MAb BT-1 increased the frequency of conjugate formation ~2-fold but had no effect on VS formation, consistent with the observation that ICAM-2 was not enriched at the synapse. There was no significant inhibition of either conjugate or VS formation when the nonblocking MAb TS2/4 or MHM24 was present, implying that inhibitory effects are epitope specific and are unlikely to be due solely to steric interference.

These data reveal a complex pattern of inhibitory events in

TABLE 2. Effects of adhesion molecule-specific antibodies and cyclic peptides on VS formation

Treatment	% Formation (SE)[a]		n^d
	Conjugate[b]	VS[c]	
Untreated control	19 (3)	46 (6)	258
Anti-ICAM-1 (LB-2)	17 (2)	32*[e] (6)	448
Anti-ICAM-2 (BT-1)	37* (5)	31 (8)	128
Anti-ICAM-3			
101-1D2	22 (2)	34* (10)	222
BRIC79	7** (3)	13* (9)	198
Anti-LFA-1 α_L			
25.3.1	17 (4)	8** (5)	226
L15	19 (4)	27** (9)	309
TS2/4	15 (3)	27 (8)	157
MHM24	23 (3)	39 (5)	101
Anti-LFA-1 β_2			
MHM23	41** (9)	5** (4)	198
L130	11* (3)	17* (9)	210
Peptide control	27 (3)	19 (5)	219
cIBR peptide	17* (2)	15 (6)	291
cLAB.L peptide	15** (2)	16 (5)	332

[a] Standard errors are from replicate experiments. Statistical analysis was performed using nonparametric tests with Bonferroni's correction for multiple comparisons. Asterisks indicate results significantly different from those for the untreated control. *, $P < 0.05$; **, $P < 0.01$.

[b] Mean percentage of primary $CD4^+$ target T cells in conjugates with $Jurkat_{IIIB}$ effector cells, counted from randomly chosen fields.

[c] Mean percentage of conjugates that showed copolarization of CD4, Env, and Gag to the cell-cell interface.

[d] Number of primary cells examined from randomly chosen low-power fields.

which MAbs interfered with both conjugate and VS formation (L130 and BRIC79) or with VS assembly alone (25.3.1, L15, 101-1D2, LB-2). Other MAbs increased the frequency of conjugate formation (BT-1, MHM23) while reducing that of VS formation (MHM23). To further exclude nonspecific inhibition of conjugate and VS formation by MAbs, we tested cyclic peptides designed to inhibit LFA-1–ICAM interactions (14, 35) for their effects on these processes. Target $CD4^+$ T cells were preincubated with the cIBR or cLAB.L peptide at 37°C, mixed with effector cells, incubated for 1 h, fixed, and processed for LSCM. Both the cIBR and cLAB.L peptides significantly reduced the frequency of formation of conjugates (40%) between target and effector cells from that with the peptide control but failed to inhibit VS formation in the remaining conjugates (Table 2).

Although it is evident from these data that adhesion interactions are functional in T-cell conjugate formation and VS assembly, the complex pattern of modulation by the inhibitors is difficult to interpret. Our data imply that the stabilization of T-cell–T-cell conjugates by adhesion interactions is dissociable from VS formation, suggesting a more complex role for these molecules than simply cell-cell adhesion. Thus, MAbs that interfere with VS assembly without affecting (LB-2, 25.3.1, L15) or while increasing (MHM23) conjugate frequency may induce signaling within T cells, inhibiting the recruitment of receptors on the target cell, that of Env on the effector cells, or conceivably both. The reduction of conjugate frequency by the cyclic peptides in the absence of a reduction in VS assembly implies that the peptides may simply be reducing the stability of cell-cell adhesion without inducing or altering adhesion molecule-mediated signaling to the VS. A second, nonexclusive explanation is that LFA-1–ICAM-3 interactions are more important than LFA-1–ICAM-1 interactions in conjugate and VS formation when CXCRX4-tropic viruses infect naïve or resting $CD4^+$ $CXCR4^+$ T cells and that the peptides more effectively inhibit the binding of LFA-1 to ICAM-1 than to ICAM-3. Since the cIBR peptide sequence is derived from the ICAM-1 D1 domain (14), it is unlikely to inhibit LFA-1–ICAM-3 binding as efficiently as LFA-1–ICAM-1 binding, especially given the fact that a much larger region of the ICAM-3 D1 domain contributes to LFA-1 association (2). Furthermore, the cLAB.L peptide is derived from the LFA-1 I domain and has been reported to bind less well to ICAM-3 than to ICAM-1 (35).

To further define the role of LFA-1 in VS assembly and function, we incubated $Jurkat_{IIIB}$ cells with target Jurkat cells—either the wild-type parental control (Jn9) or cells that do not express LFA-1 ($\beta_{2.7}$) or that express LFA-1 trapped in a low-affinity (lo1.3) or high-affinity (hi1.19) conformation (6, 33)—for various times and quantified VS formation (Fig. 2A). After 1 h, >30% of parental Jn9 cells had formed a VS with $Jurkat_{IIIB}$ cells, whereas only 10% of target cells that did not express LFA-1 or that expressed the low-affinity or high-affinity conformational mutant had formed a VS ($P = 0.017$, $P = 0.05$, and $P = 0.004$, respectively). Prolonged incubation did not increase the number of VSs that we detected, in agreement with our earlier observations that VS formation achieves maximal levels within 1 h (19). Representative images (Fig. 2B) show that VSs between $Jurkat_{IIIB}$ cells and target cells expressing mutated LFA-1 are microscopically indistinguishable from those seen with the parental cell line.

We have developed a quantitative real-time PCR (qRT-PCR) assay that reports direct cell-cell transfer of HIV-1 by assaying de novo synthesis of HIV-1 *pol* reverse transcripts expressed in relation to invariant human serum albumin (HSA) DNA (20). Detection of new viral DNA demonstrates reverse transcription of incoming viral RNA genomes, a proportion of which would be expected to integrate into the host genome. Using this system, we compared Jn9 Jurkat cells with $\beta_{2.7}$, hi1.19, and lo1.3 mutants as targets for VS formation with $Jurkat_{IIIB}$ effectors (Fig. 2C). A time-dependent increase in HIV-1 DNA transcript levels was seen in Jn9 cells, reaching ~6-fold above background after 6 h. By contrast, the LFA-1-$\beta_{2.7}$ cells yielded a 1.5-fold-lower signal at 6 h ($P = 0.009$). A similar trend was observed for the high-affinity mutant ($P = 0.017$), consistent with the report that cells expressing constitutively active LFA-1 are less able to establish spreading viral infections (15). Interestingly, the low-affinity mutant showed a trend toward increased viral DNA synthesis (~9-fold above background at 6 h), although this level was not significantly different from that for the parental Jn9 cells ($P = 0.237$). These differences were not due to variable expression of CD4 or CXCR4 on the different Jurkat target cells; these levels were very similar, as determined by immunofluorescent staining and flow cytometry (results not shown).

The inhibition and mutant cell line data, taken together, indicate that LFA-1–ICAM interactions contribute both to maintaining stability at the T-cell–T cell interface and to pro-

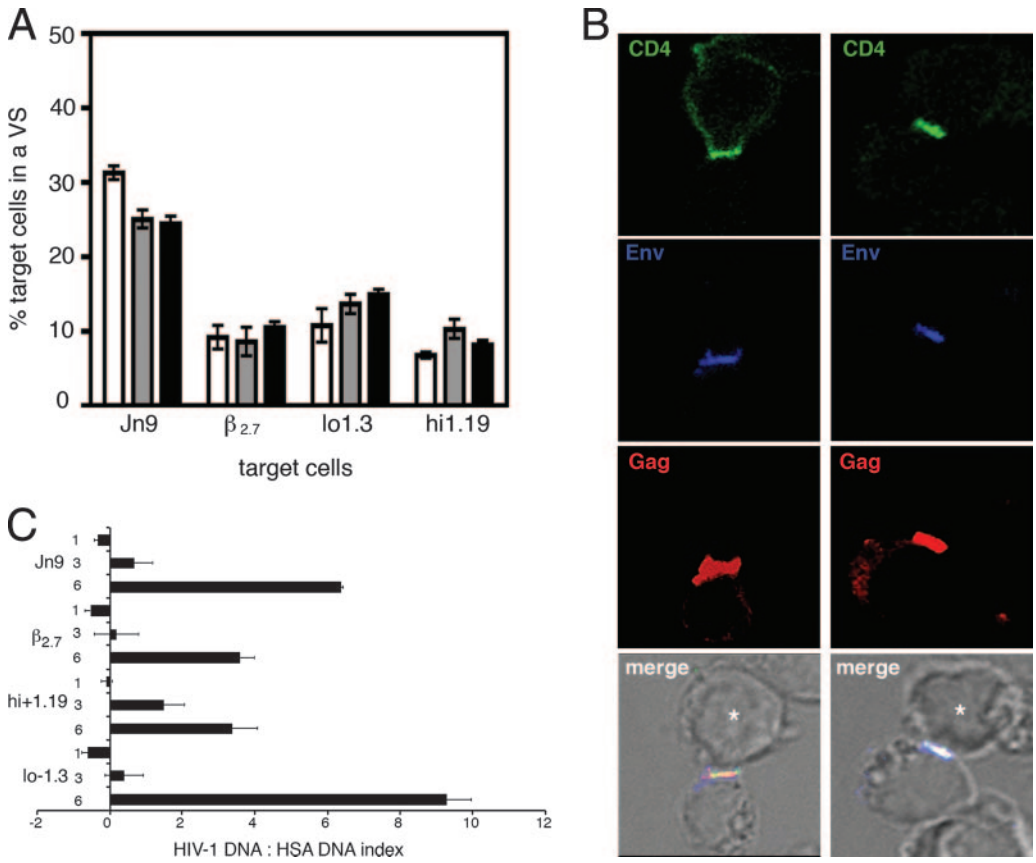

FIG. 2. LFA-1 mutant T cells show impaired VS formation. (A) $Jurkat_{IIIB}$ cells were incubated with an equal number of uninfected parental Jn9 (wild-type LFA-1), $\beta_{2.7}$ (no LFA-1), lo1.3 (low-affinity LFA-1), or hi1.19 (high-affinity) target cells (a gift from Lloyd Klickstein, Brigham and Women's Hospital, Boston, MA) (6, 33) for 1 h (open bars), 3 h (shaded bars), or 6 h (solid bars) at 37°C. At each time point, the cells were fixed and stained for CD4, Env, and Gag. Multiple random low-power fields were selected, and target cells forming VSs were counted. Bars represent the mean percentage (from two independent experiments) of target cells that formed VSs at each time point for each target cell type. Error bars, standard errors of the means. (B) Representative immunofluorescence images taken through the middle of conjugates formed between $Jurkat_{IIIB}$ cells and target T cells (asterisks), either wild-type Jn9 (left) or LFA-1-deficient $\beta_{2.7}$ (right) cells. LFA-1-expressing and non-LFA-1-expressing cells show similar CD4 (green), Env (blue), and Gag (red) staining, and both form a VS. (C) qRT-PCR was performed to measure cell-cell spread of HIV-1. $Jurkat_{IIIB}$ effector cells were mixed with an equal number of target cells, either Jn9, $\beta_{2.7}$, lo1.3, or hi1.19 cells, and incubated at 37°C prior to lysis at 0, 1, 3, or 6 h. Total genomic DNA was extracted immediately, and qRT-PCR using *pol* primers was performed to measure de novo viral DNA synthesis in Jn9 cells and LFA-1 mutants as described elsewhere (20). Data were normalized to those for HSA and are expressed as the ratio of HIV-1 DNA to HSA DNA. The baseline signal obtained at 0 h was subtracted. Error bars, standard errors of the means of replicate experiments.

moting VS assembly and cell-cell spread. Stable conjugate formation would provide an environment in which other receptor interactions would be enhanced and HIV-1 could transfer across the synapse. However, data from our MAb inhibition studies also suggest that adhesion interactions may influence VS formation independently of conjugate formation. This is consistent with the ability of LFA-1 and the ICAMs to regulate immunological functions including coordination of chemotaxis, cell migration, and activation of T cells at the IS (16, 29). Of particular importance, signaling via LFA-1 and the ICAMs can modify the underlying cytoskeleton, resulting in receptor recruitment and increased IS stability (3, 26, 29). Consistent with this, cross-linking of ICAM-1 and, to a lesser extent, LFA-1 on activated effector T cells has been shown to facilitate VS formation by HTLV-1 (1, 23), most likely by induction of signaling cascades leading to polarization of the effector cell cytoskeleton.

Here we demonstrate that ICAM-3 is enriched at the VS and functions in effector–target cell conjugate stabilization, leading to cell-cell transfer of HIV-1. By contrast, in the system that we are using, LFA-1–ICAM-1 binding appears to be less important in HIV-1 VS formation or cell-cell spread. This finding contrasts with studies on cell-free infection by HIV-1 showing that ICAM-1 enhances viral adhesion and infection (10–12, 24, 27, 31). This difference is probably explained by the target cell types used: we used resting primary $CD4^+$ $CXCR4^+$ T cells expressing high levels of ICAM-3 but low levels of ICAM-1, whereas other studies used immortalized or activated T cells or lines expressing high levels of ICAM-1. Since memory $CD4^+$ T cells are $CD4^+$ $CCR5^+$ and express higher levels of ICAM-1, ICAM-1 may play a more central role in cell-cell spread by CCR5-tropic strains of HIV-1. Future studies will be aimed at characterizing the function of adhesion molecules beyond adhesion in promoting VS assembly and investigating

whether these interactions are also implicated in the T-cell-to-T-cell transmission of CCR5-tropic HIV-1.

We thank the many individuals and groups who kindly provided antibodies and other reagents used in this study. We thank Jonathan Marchini for assistance with statistical analysis.

This work was funded by grant G0400453 from the Medical Research Council of the United Kingdom.

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